REMARKS

Claims 1-37 are pending and have been rejected. Claims 15, 28, and 30-37 are withdrawn from consideration. Claims 1, 6, 8, 16, 19, 22, 30 and 33 have been amended. Claims 3 and 20 have been canceled. Claims 38-55 have been added. Support for the amendments and new claims can be found in original claims 3 and 20, and the disclosure at Examples 2, 3, 4 and 6. Claims 1, 2, 4-19, and 21-55 remain in the case.

The Examiner acknowledges applicants' election of Group I and the species DOTA-bearing peptides, and states that applicants did not traverse the requirement. Applicants did, however, note that elected Group I includes product claims, and that once the elected product claims are found allowable, that method claims should be rejoined and examined. Applicants have amended the nonelected method claims to ensure that the recited product encompasses the same scope as the elected product claims.

The disclosure is objected to for failing to recite that it claims the benefit of the earlier-filed provisional application. The Examiner additionally requests that applicants update the status of U.S. applications cited in the specification. These points have been addressed.

Claims 1-14 and 16-18 are rejected under the second paragraph of Section 112. The Examiner urges that the method claims are incomplete, for not clearly setting forth method steps and not including a resolution step that relates back to the preamble. The method claims have been amended to address these points.

The Examiner further objects to the phrase "ketone derivative of a saccharide or saccharide precursor," urging that the exact meaning of the phrase is unclear because there is not universally accepted meaning for this term and it is not adequately defined in the specification. More particularly, the Examiner argues that "there is no way for a person of skill in the art to ascribe a discrete and identifiable class of compounds to said phrase." However, the specification

provides an adequate definition and guidance to skilled artisan, noting that the method can employ:

> a ketone derivative of a saccharide (such as N-levulinoyl fucose) or saccharide precursor (such as N-levulinoyl mannosamine (ManLev)), resulting in an antibody comprising reactive ketone groups at the N-glycosylation sites. In the case of ManLev, biosynthetic pathways convert the ManLev to levulinoyl sialic acid, which is incorporated into the antibody at the glycosylation site. In the case of N-levulinoyl fucose, the N-levulinoyl fucose itself is incorporated into the antibody at the glycosylation site.

Accordingly, reconsideration and withdrawal of this basis for rejection under the second paragraph of Section 112 is respectfully requested.

Finally, the Examiner objects to the abbreviation of DOTA at its first appearance in the claims. The claims have been amended to provide the full name for DOTA and DTPA at their first occurrence.

Claims 1-14 and 16-18 are rejected under the first paragraph of Section 112. The Examiner urges that claims of this scope are not enabled for methods of making glycosylated antibodies or immunoconjugates other than antibodies comprising hLL2 having a reactive ketone group on the on the glycosylated sites in HCN1, HCN5 and position 18 in Vκ ("Vκ-N"), wherein the expressed antibody is expressed in SP2/0 cells in a culture medium comprising N-levulinoyl mannosamine and N-levulinoyl fucose. Applicants have amended the independent claims to recite methods in which SP2/0 cells are transfected with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or Vκ domain. It is well within the level of skill in this art to use glycosylation sites other than HCN1, HCN5 and Vκ-N. As noted on page 8 of the specification, "additional glycosylation sites can be identified and engineered in the Vκ, VH, CH1 CH2, CH3 and Cκ domains in accordance with procedures known to those skilled in the art." More particularly, page 7 of the specification teaches that "antibodies genetically engineered to have one or more N-glycosylation sites are preferred for landscaping in accordance with the present invention. Such antibodies can be made in accordance with known procedures. U.S.

Provisional Patent Application 60/013,709, the contents of which are incorporated herein by reference in their entirety, describes suitable multiply glycosylated antibodies."

Example 2 provides guidance for the selection and screening of glycosylation sites, and describes the design of a variety of sites. Sites were designed that are: (1) naturally found in the constant domains of other antibodies, (2) at a surface position as identified by computer modeling, or (3) randomly selected sites "evenly" dispersed along the Cκ and CH1 domains, in order to identify proper positions for efficient glycosylation. A total of five CH1 sites (HCN1-5) and five Cκ sites (KCN1-5) were designed and engineered in Example 2. To engineer the novel CHO moieties, the glycosylation acceptor sequences, Asn-X-Ser/Thr, were introduced into the Cκ and CH1 domains of hLL2 by site-specific mutagenesis. In all cases except for KCN5, possible perturbations in the final tertiary structure were minimized by carefully choosing sequences that required only single amino acid substitution to become a potential glycosylation site. In the case of KCN5, two amino acid residues were changed to form the sequence Asn-Val-Ser (166-168).

Computer modeling can assist in the design of engineered glycosylation sites. For example, Example 2 notes that none of the designed sites appeared to be "buried" or at the interface between two juxtaposed domains, as evaluated by computer modeling analyses. The determination of whether the engineered sites were efficiently glycosylated is straightforward. The antibody mutants were purified from stably transfected cells and analyzed in SDS-PAGE under reducing conditions. The heavy chains of the mutant antibodies hLL2HCN1-5 migrated at slower rates, due to glycosylation at the engineered sites, compared to that of the parent antibody, hLL2, whose CH1 domain did not contain any potential glycosylation sites. From the relative migration rates of the peptides in SDS-PAGE, which are inversely proportional to the molecular sizes, the extent of glycosylation at the different sites was estimated to be HCN5 > HCN1 > HCN3 > HCN2 > HCN4, with hLL2HCN5 and hLL2HCN1 being the two most highly glycosylated antibodies. In contrast, the lack of migration retardation in the light chains for the mutants hLL2KCN1-5, led to the conclusion that these Cκ-associated sites were not glycosylated. A skilled artisan also would be able to use ketone derivatives of saccharides and saccharide

precursors other than N-levulinoyl mannosamine and N-levulinoyl fucose, which are merely exemplary embodiments of useful ketone derivatives.

It also was within the level of skill in this art to use antibodies other than hLL2 antibodies. For example, U.S. 5,635,603 describes the introduction of an Asn-linked glycosylation site in the VK FR1 Region of humanized MN14:

> An Asn-glycosylation site was introduced into the VK FR1 region of humanized MN14, which is an antibody that binds carcinoembryonic antigen. Briefly, the nucleotide sequence encoding Arg_{18} was mutated to a nucleotide sequence encoding Asn_{18} using the PCR method described in Example 1. In this case, DNA from the light chain expression vector for humanized MN14 was used as a template for PCR. The VKFOR1 primer of Orlandi *et al.* was used as the 3' primer. The 5' primer consisted of a 57-mer encoding the first 20 amino acids of the MN14 VK domain, with the exception that the codon at position 18 encoded Asn. The approximately 300 base pair PCR product was digested with PvuII and BglII, and ligated into complementary sites in a staging or cloning vector. DH5.alpha. competent cells were transformed with the staging or cloning vector using a standard calcium chloride method. For example, see, Ausubel *et al., supra.*
>
> The DNA fragment containing the humanized MN14 VK sequence with an Asn-glycosylation site at amino acid position 18 was subcloned into a pSVhyg-based light chain expression vector. SP2/0-AG14 non-secreting myeloma cells were co-transfected by electroporation with the linearized light chain expression vector and with a linearized heavy chain expression vector. Transfectomas were selected using hygromycin-B and cultured to produce antibody.
>
> Antibody was purified and analyzed on an SDS-PAGE reducing gel. The light chain of the glycosylated humanized MN14 migrated as multiple bands and ran at a higher molecular weight, compared to non-glycosylated MN14 light chain. This result indicates that the new Ash-linked glycosylation site was used for carbohydrate addition.
>
> Significantly, the MN14 blocking activities of the glycosylated MN14 antibody and the non-glycosylated MN14 antibody were found to be substantially the same. Thus, glycosylation at the VK FR1 region of humanized MN14 does not affect immunoreactivity.

Accordingly, the design and testing of CH1 and $V\kappa$ N-glycosylated sites, in LL2 or other antibodies, and the production of antibodies with culture media containing a variety of ketone

derivatives is enabled by the level of skill in this art and by teachings found within applicants' specification.

Claims 19, 21, 22, 24-26 and 29 are rejected under Section 102(b) based on Sivam (WO 90/03401). The Examiner directs applicants' attention to pages 2 and 3 of Sivam. The identified portion describes the generation of an aldehyde on an immunoglobulin molecule, which is reacted with a hydrazide to form a hydrazone. Page 2 describes the generation of aldehyde groups by oxidizing oligosaccharide moieties on an immunoglobulin molecule, and their reaction with a primary amine to form a Schiff base. However, it is noted that the harsh oxidation used to generate the aldehyde groups, especially where complete oxidation of all carbohydrate residues is desired, and the harsh reducing environment used to stabilize the Schiff base conjugate, both may impair the biological activity of the molecule. Alternatively, the aldehyde generated on an immunoglobulin may be reacted with a hydrazide to form a hydrazone. But, as noted on page 3 of Sivam, this method still suffers from several disadvantages: (1) the immunoglobulin must still be oxidized to generate free aldehyde groups; and (2) the degree of conjugation is limited by the number of carbohydrate moieties present on the immunoglobulin molecule. By contrast, the antibodies according to the present invention have a reactive ketone group, which is produced by the transfected host cell's biosynthetic machinery. Sivam does not disclose a glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on the glycosylated site. In particular, Sivam does not disclose such a glycosylated antibody prepared by the method of claim 1.

Claims 22-26 are rejected under Section 102(b) based on Leung *et al., J. Immunol.* 154: 5919 (1995). As in Sivam, in order to conjugate at these carbohydrates, the ribose rings must be chemically oxidized to generate reactive aldehyde groups. Aldehyde groups thus formed can be covalently bonded to the amino groups of chelates or drugs through Schiff bases. Since only the C-C bonds with hydroxyl groups attached to each carbon can be periodate-oxidized to form two aldehyde groups, the maximum number of these reactive sites is dictated by the structure and linkages of the oligosaccharide.

For example, the compositions and sequences of CH1-appended carbohydrates from two antibodies, hLL2HCN1 and hLL2HCN5, have been determined by fluorophore-assisted carbohydrate electrophoresis (FACE) 16411. Qu *et al.*, Glycobiol. 7(6): 803-09 (1997). The structural profile of hLL2HCN1-carbohydrates revealed that about 2-4 hexose rings in an oligosaccharide chain are available for periodate oxidation. Therefore, a maximum of 8-16 aldehyde groups on average can be generated from the carbohydrate side chains of each hLL2HCN1 $F(ab')_2$ fragment. With the average size of hLL2HCN5-carbohydrate being 3-4 monosaccharide residues larger than that of HCN1, a higher number of maximum achievable aldehyde groups for hLL2HCN5 is expected. Under mild chemical conditions, only 1.6 and 3 molecules of DTPA were conjugated to the $F(ab')_2$ of hLL2HCN1 and hLL2HCN5 sites, respectively, probably due to inefficient oxidation of hexose rings under these conditions. Although harsher conditions can be used to generate more aldehyde groups, they may alter the three-dimensional structure of the antibodies and the immunoreactivities of the antibodies may suffer. As noted above, the antibodies according to the present invention have a reactive ketone group, which is produced by the transfected host cell's biosynthetic machinery. Leung *et al.* does not disclose a glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, and more particularly a glycosylated antibody prepared by the method of claim 1.

Claims 19-21 are rejected under Section 103(a) based on Sivam in view of Hansen (U.S. 5,443,953). The failings of Sivam are discussed above. Hansen describes the introduction of multiple glycosylation sites on the Vκ and CH1 (HCN1 and HCN5 sites) domains of antibodies. It does not overcome the failure of Sivam to suggest glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, and more particularly a glycosylated antibody prepared by the method of claim 1.

Claims 22-25 and 27 are rejected under Section 103(a) based on Sivam in view of Li *et al.* The failings of Sivam are discussed above. Li *et al.* describes peptide-linked DOTA derivatives and corresponding radiolabeled immunoconjugates. It does not overcome the failure of Sivam to suggest glycosylated antibody or antigen-binding antibody fragment having a reactive ketone

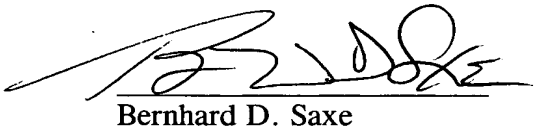
group on the glycosylated site, and more particularly a glycosylated antibody prepared by the method of claim 1.

In summary, none of the cited references teach glycosylated antibodies or antibody fragments which have reactive ketone groups on the glycosylated sites. In accordance with the present invention, these antibodies are made recombinantly by a transfected host cell. The host cell's biosynthetic machinery converts the antibodies so that they have a reactive ketone group. Reconsideration and withdrawal of the rejections under Sections 102 and 103 based on Sivam, Leung, Hansen and Li is respectfully requested.

Claims 22-26 are rejected under the doctrine of obviousness-type double patenting based on Hansen. As discussed above, Hansen describes the introduction of multiple glycosylation sites on the Vκ and CH1 (HCN1 and HCN5 sites) domains of antibodies. It does not suggest glycosylated antibodies or antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, and more particularly glycosylated antibodies prepared by the method of claim 1. Reconsideration and withdrawal of this rejection based on Hansen is respectfully requested.

Based on the foregoing amendments and remarks, all claims are believed to be in condition for allowance. Should there be any matter requiring further attention, the Examiner is invited to contact the undersigned at the local telephone exchange listed below.

Respectfully submitted,



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Date

Bernhard D. Saxe
Reg. No. 28,665

FOLEY & LARDNER
3000 K Street, N.W., Suite 500
Washington, D.C. 20007-5109
Telephone: (202) 672-5427
Facsimile: (202)672-5399

MARKED-UP VERSIONS OF CLAIM AMENDMENTS

1. (Amended) A method of making a glycosylated antibody having a reactive ketone group on the glycosylated site, comprising:

[expressing a cell transfected] transfecting SP2/0 cells with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or Vκ domain in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor, and

expressing said transfected SP2/0 cells so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site.

6. (Amended) A method of making a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, comprising:

[expressing a cell transfected] transfecting SP2/0 cells with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or Vκ domain in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor,

expressing said transfected SP2/0 cells so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site, and

fragmenting the resulting glycosylated antibody into [an] a glycosylated antigen-binding antibody fragment having a ketone group on the glycosylated site.

8. (Amended) A method of making an immunoconjugate comprising a glycosylated antibody conjugated to an agent through its glycosylated site, comprising:

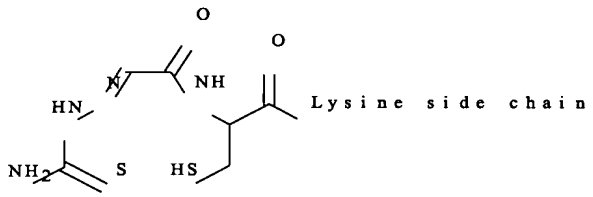
[expressing a cell transfected] transfecting SP2/0 cells with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or Vκ domain in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor,

expressing said transfected SP2/0 cells so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site,

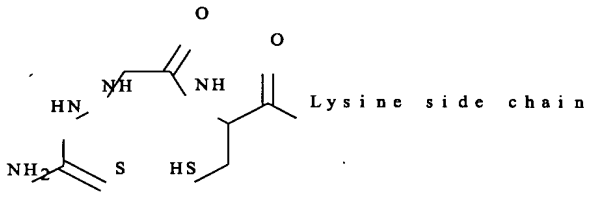
reacting the resulting antibody with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, and

conjugating said glycosylated antibody to an agent through its glycosylated site.

14. The method of claim 8, wherein the agent is a ligand-containing peptide selected from the group consisting of diethylene triamine pentaacetic acid-bearing (DTPA-bearing) peptides, 1,4,7,10-tetraazacyclododecane-N,N',N"N'"-tetraacetic acid-bearing (DOTA-bearing) peptides, $AcK_dD_dK_d(TscGC)D_dK_d$-$NH(CH_2)_4CH(NH_2)CONH$-NH_2, $AcK_dD_dK_d(TsdGC)D_dK_d$-$NH(CH_2)_4H(NH_2)CONH$-NH_2, and H_2N-NH-CH_2-CO-D_d-$K_d(TscGC)$-D_d-K_d-NH_2, where K_d and D_d represent the D-amino acids D-lysine and D-aspartic acid, respectively, and where TscGC is the ligand:



and TsdGC is the ligand:



16. (Amended) A method of making an immunoconjugate comprising a glycosylated antigen-binding antibody fragment conjugated to an agent through the glycosylated site, comprising:

[expressing a cell transfected] transfecting SP2/0 cells with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or V_K domain in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor,

expressing said transfected SP2/0 cells so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site,

fragmenting the resulting antibody into an antigen-binding antibody fragment, and

reacting the antibody fragment with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides.

19. (Amended) A glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on [the] a glycosylated site, wherein said glycosylated site is in the Vκ or the CH1 domain.

20. (Amended) The glycosylated antibody or antigen-binding antibody fragment of claim 19, wherein [the antibody or antibody fragment is glycosylated in a domain selected from the group consisting of the Vκ domain and the CH1 domain] said glycosylated site is selected from the group consisting of HCN1, HCN5 and Vκ-N.

22. (Amended) An immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment conjugated to an agent through [the] a reactive ketone on a glycosylated site, wherein said glycosylated site is in the Vκ or CH1 domain.

23. (Amended) The immunoconjugate of claim 22, wherein the glycosylated site is [in a domain selected from the group consisting of the Vκ domain and the CH1 domain] selected from the group consisting of HCN1, HCN5 and Vκ-N.

30. (Amended) A method of targeting an active agent to an *in vivo* target site comprising administering an immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment conjugated to an active agent through the or antigen-binding antibody fragment conjugated to an active agent through [the] a glycosylated HCN1, HCN5 or Vκ-N glycosylation site.

33. (Amended) A method of targeting an active agent to an *in vivo* target site comprising:

administering a glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on [the] a HCN1, HCN5 or Vκ-N glycosylation site, and allowing the antibody or antibody fragment to localize at the target site;

optionally, administering a clearing agent to clear non-localized antibody or antibody fragment from circulation; and

administering an active agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, whereby the active agent localizes at the target site via conjugation with the pre-targeted antibody or antibody fragment.